

Conditions for cell size homeostasis: A stochastic hybrid systems approach

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A ubiquitous feature of living cells is their growth over time followed by division into daughter cells. How isogenic cell populations maintain size homeostasis, i.e., a narrow distribution of cell size, is an intriguing fundamental problem. We model cell size using a stochastic hybrid system, where a cell grows exponentially in size (volume) over time and probabilistic division events are triggered at discrete time intervals. Moreover, whenever division events occur, size is randomly partitioned among daughter cells. We first consider a scenario, where a timer (i.e., cell-cycle clock) that measures the time since the last division event regulates both the cellular growth and division rates. Analysis reveals that such a timer-controlled system cannot achieve size homeostasis, in the sense that, the cell-to-cell size variation grows unboundedly with time. To explore biologically meaningful mechanisms for controlling size we consider two classes of regulation: a size-dependent growth rate and a size-dependent division rate. Our results show that these strategies can provide bounded intercellular variation in cell size, and exact mathematical conditions on the form of regulation needed for size homeostasis are derived. Different known forms of size control strategies, such as, the adder and the sizer are shown to be consistent with these results. Interestingly, for timer-based division mechanisms, the mean cell size depends on the noise in the cell-cycle duration but independent of errors incurred in partitioning of volume among daughter cells. In contrast, the mean cell size decreases with increasing partitioning errors for size-based division mechanisms. Finally, we discuss how organisms ranging from bacteria to mammalian cells have adopted different control approaches for maintaining size homeostasis.

Index Terms—Cell size homeostasis; Stochastic hybrid systems; Moment closure

I. INTRODUCTION

Stochastic hybrid systems (SHS) constitute an important mathematical modeling framework that combines continuous dynamics with discrete stochastic events. Here we use SHS to model a universal feature of all living cells: growth in cell size (volume) over time and division into two viable progenies (daughters). A key question is how cells regulate their growth and timing of division to ensure that they do not get abnormally large (or small). This problem has been referred to literature as *size homeostasis* and is a vigorous area of current experimental research in diverse organisms [1–16]. We investigate if phenomenological models of cell size dynamics based on SHS can provide insights into the control mechanisms needed for size homeostasis.

The proposed model consists of two non-negative state variables: $v(t)$, the size of an individual cell at time t , and a timer τ that measures the time elapsed from when the cell was born (i.e., last cell division event). This timer can be biologically interpreted as an internal clock that regulates cell-cycle processes. Time evolution of these variables is governed by the following ordinary differential equations

$$\dot{v} = \alpha(v, \tau)v, \quad \dot{\tau} = 1, \quad (1)$$

where the *growth rate* $\alpha(v, \tau) \geq 0$ can depend on both state variables and is such that (1) has a unique and well-defined solution $\forall t \geq 0$ (i.e., cell size does not blow up in finite time). A constant α implies exponential growth over time.

As the cell grows in size, the probability of cell division occurring in the next infinitesimal time interval $(t, t + dt]$ is given by $f(v, \tau)dt$, where $f(v, \tau)$ can be interpreted as

the *division rate*. Whenever a division event is triggered, the timer is reset to zero and the size is reduced to βv , where random variable $\beta \in (0, 1)$ is drawn from a beta distribution. Assuming symmetric division, β is on average half, and its coefficient of variation (CV_β) quantifies the error in partitioning of volume between daughters. To be biologically meaningful, $\alpha(v, \tau)$ is a non-increasing function, while $f(v, \tau)$ is a non-decreasing function of its arguments. The SHS model is illustrated in Fig. 1 and incorporates two key noise sources: randomness in partitioning and timing of division. Next, we explore conditions for size homeostasis, in the sense that, the mean cell size does not converge to zero, and all statistical moments of v remain bounded.

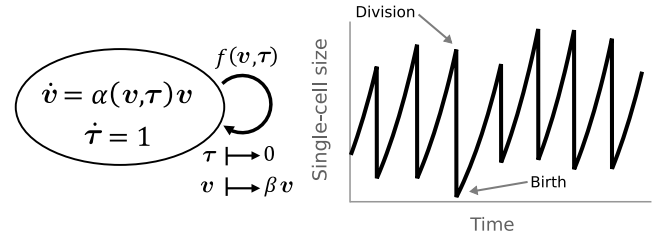


Fig. 1: SHS model for capturing time evolution of cell size. The size of an individual cell $v(t)$ grows exponentially with growth rate $\alpha(v, \tau)$, where τ represents a timer that measures the time since the last division event. The arrow represents cell division events that occur with rate $f(v, \tau)$, which resets τ to zero and divide the size by approximately half. A sample trajectory of $v(t)$ is shown with cycles of growth and division.

II. TIMER-DEPENDENT GROWTH AND DIVISION

We begin by considering a scenario, where both the growth and division rates are functions of τ , but do not depend on v .

The SHS can be compactly written as

$$\dot{\mathbf{v}} = \alpha(\tau)\mathbf{v}, \quad \dot{\tau} = 1, \quad (2)$$

with reset maps

$$\mathbf{v} \mapsto \beta\mathbf{v}, \quad \tau \mapsto 0 \quad (3)$$

that are activated at the time of division. The timer-controlled division rate $f(\tau)$ can be interpreted as a ‘‘hazard function’’ [17]. Let T_1, T_2, \dots denote independent and identically distributed (i.i.d.) random variables that represent the time interval between two successive division events. Then, based on the above formulation, the probability density function (pdf) for T_i is given by

$$T_i \sim f(x)e^{-\int_{y=0}^x f(y)dy}, \quad \forall x \geq 0 \quad (4)$$

[17]. Note that a constant division rate in (4) would lead to an exponentially distributed T_i . For this class of models, the steady-state statistics of \mathbf{v} is given by the following theorem.

Theorem 1: Consider the SHS (2)-(3) with timer-dependent growth and division rates. Then

$$\lim_{t \rightarrow \infty} \langle \mathbf{v}(t) \rangle = \begin{cases} 0 & \left\langle e^{\int_{y=0}^{T_i} \alpha(y)dy} \right\rangle < 2 \\ \infty & \left\langle e^{\int_{y=0}^{T_i} \alpha(y)dy} \right\rangle > 2, \end{cases} \quad (5)$$

where the symbol $\langle \cdot \rangle$ is used to denote the expected value of a random variable. Moreover,

$$0 < \lim_{t \rightarrow \infty} \langle \mathbf{v}(t) \rangle < \infty, \quad \lim_{t \rightarrow \infty} \langle \mathbf{v}^2(t) \rangle = \infty \quad (6)$$

when $\left\langle e^{\int_{y=0}^{T_i} \alpha(y)dy} \right\rangle = 2$. ■

Proof of Theorem 1: Let \mathbf{v}_{i-1} denote the cell size just at the start of the i^{th} cell cycle. Using (2), the size at the time of division in the i^{th} cell cycle is given by

$$\mathbf{v}_{i-1} e^{\int_{y=0}^{T_i} \alpha(y)dy}. \quad (7)$$

Thus, the size of the newborn cell in the next cycle is

$$\mathbf{v}_i = \mathbf{v}_{i-1}\mathbf{x}_i, \quad \mathbf{x}_i := \beta_i e^{\int_{y=0}^{T_i} \alpha(y)dy}, \quad (8)$$

where $\beta_i \in (0, 1)$ are i.i.d random variables following a beta distribution and \mathbf{x}_i are i.i.d. random variables that are a function of β_i and T_i . From (8), the mean cell size at the start of i^{th} cell cycle is given by

$$\langle \mathbf{v}_i \rangle = \mathbf{v}_0 \langle \mathbf{x}_i \rangle^i \quad (9)$$

and will grow unboundedly over time if $\langle \mathbf{x}_i \rangle > 1$, or go to zero if $\langle \mathbf{x}_i \rangle < 1$. Using the fact that $\langle \beta_i \rangle = 0.5$ (symmetric division of a mother cell into daughter cells), β_i and T_i are independent, (5) is a straightforward consequence of (9). It also follows from (8) that

$$\langle \mathbf{v}_i^2 \rangle = \mathbf{v}_0^2 \langle \mathbf{x}_i^2 \rangle^i = \mathbf{v}_0^2 \langle \mathbf{x}_i \rangle^{2i} (1 + CV_{\mathbf{x}_i}^2)^i \quad (10)$$

where $CV_{\mathbf{x}_i}^2$ represents the coefficient of variation squared of \mathbf{x}_i . When $\langle \mathbf{x}_i \rangle = 1$ then $\langle \mathbf{v}_i \rangle = \mathbf{v}_0$ and

$$\langle \mathbf{v}_i^2 \rangle = \mathbf{v}_0^2 (1 + CV_{\mathbf{x}_i}^2)^i. \quad (11)$$

Note that when the system is completely deterministic, i.e., pdfs for T_i and β_i are given by delta functions, $CV_{\mathbf{x}_i}^2 = 0$. However, the slightest noise in these variables will lead to $CV_{\mathbf{x}_i}^2 > 0$, in which case (11) implies (6). ■

In summary, unless functions $\alpha(\tau)$ and $f(\tau)$ are chosen such that $\left\langle e^{\int_{y=0}^{T_i} \alpha(y)dy} \right\rangle = 2$, the mean cell size would either grow unboundedly or go extinct. Moreover, even if the mean cell size converges to a non-zero value, the statistical fluctuations in size would grow unboundedly. Hence, size-based regulation of growth/division rates is a necessary condition for size homeostasis.

III. SIZE-DEPENDENT GROWTH RATE

Recent work measuring sizes of single mammalian cells over time has reported lowering of growth rates as cells become bigger [18–20]. To explore the effects of such regulation, we consider a growth rate $\alpha(\mathbf{v}, \tau)$ that now depends on size. As in the previous section, timer-controlled division events occur with rate $f(\tau)$ resulting in inter-division times T_i given by (4). The following result shows that size homeostasis is possible if growth rate is appropriately bounded from below and above.

Theorem 2: Let the growth rate be bounded by

$$\alpha(\mathbf{v}, \tau)\mathbf{v} \leq k(\tau)\mathbf{v}^p, \quad p \in [0, 1], \quad \forall \mathbf{v} \geq 0 \quad (12)$$

for some non-increasing function $k(\tau)$. Moreover, the growth rate of a small cell is large enough such that

$$\left\langle e^{\int_{y=0}^{T_i} \alpha_0(y)dy} \right\rangle > 2, \quad \alpha_0(\tau) := \lim_{\mathbf{v} \rightarrow 0} \alpha(\mathbf{v}, \tau). \quad (13)$$

Then

$$0 < \lim_{t \rightarrow \infty} \langle \mathbf{v}^l(t) \rangle < \left(\frac{l \langle k(\tau) \rangle \langle T_i \rangle}{\langle 1 - \beta^l \rangle} \right)^{\frac{1}{1-p}} \quad (14)$$

where $l \in \{1, 2, \dots\}$, $\langle T_i \rangle$ is the mean cell-cycle duration, and $\beta \in (0, 1)$ is a random variable quantifying the error in partitioning of volume between daughters. ■

Proof of Theorem 2: Consider a newborn cell with a sufficiently small size born at time $t = 0$. Then, the mean cell size will grow in successive generation iff the second inequality in (5) is true for $\alpha_0(\tau)$, which results in (13). Based on the Dynkin’s formula for the SHS (1) and (3), the time evolution of moments is given by

$$\frac{d\langle \mathbf{v}^l \rangle}{dt} = \langle f(\tau)\mathbf{v}^l \rangle (\langle \beta^l \rangle - 1) + l \langle \alpha(\mathbf{v}, \tau)\mathbf{v}^l \rangle, \quad (15)$$

for $l \in \{1, 2, \dots\}$ [21]. Using (12),

$$\frac{d\langle \mathbf{v}^l \rangle}{dt} \leq \langle f(\tau)\mathbf{v}^l \rangle (\langle \beta^l \rangle - 1) + l \langle k(\tau)\mathbf{v}^{l-1+p} \rangle. \quad (16)$$

Note that

$$\langle f(\tau)\mathbf{v}^l \rangle = \langle f(\tau) \rangle \langle \mathbf{v}^l | \tau \rangle \quad (17)$$

where $\langle \mathbf{v}^l | \tau \rangle$ is the expected value of \mathbf{v}^l conditioned on τ . Based on the time evolution of cell size in (1), $\langle \mathbf{v}^l | \tau \rangle$ is an increasing function of τ (cells further along in the cell

cycle, have on average, larger sizes). Since $\langle v^l | \tau \rangle$ and $f(\tau)$ are monotone non-decreasing function of τ

$$\langle f(\tau) v^l \rangle \geq \langle f(\tau) \rangle \langle v^l \rangle. \quad (18)$$

Similarly, since $k(\tau)$ is a non-increasing function,

$$\langle k(\tau) v^{l-1+p} \rangle \leq \langle k(\tau) \rangle \langle v^{l-1+p} \rangle. \quad (19)$$

Finally, using the fact that $l-1+p \leq l$ as $p \in [0, 1]$

$$\langle v^{l-1+p} \rangle = \left\langle (v^l)^{\frac{l-1+p}{l}} \right\rangle \leq \langle v^l \rangle^{\frac{l-1+p}{l}} \quad (20)$$

Using (18)-(20), (16) reduces to the following inequality

$$\frac{d\langle v^l \rangle}{dt} \leq \langle f(\tau) \rangle \langle v^l \rangle (\langle \beta^l \rangle - 1) + l \langle k(\tau) \rangle \langle v^l \rangle^{\frac{l-1+p}{l}}. \quad (21)$$

Since at steady state

$$\langle f(\tau) \rangle = \frac{1}{\langle T_i \rangle}, \quad (22)$$

[22], (21) implies (14). ■

An extreme example of size-dependent growth is

$$\alpha(v, \tau) = \frac{k}{v}, \quad k > 0 \quad (23)$$

which corresponds to cells growing linearly in size, as experimentally reported for some organisms [23]. For this case, the result below provides exact closed-form expressions for the first and second-order statistical moments of v .

Theorem 3: Consider the growth rate (23) that results in the following SHS continuous dynamics

$$\dot{v} = k, \quad \dot{\tau} = 1. \quad (24)$$

Then, the steady-state mean and coefficient of variation squared of cell size is given by

$$\begin{aligned} \lim_{t \rightarrow \infty} \langle v(t) \rangle &= \frac{k \langle T_i \rangle (3 + CV_{T_i}^2)}{2}, \\ CV_v^2 &= \frac{1}{27} + \frac{4 \left(9 \frac{\langle T_i^3 \rangle}{\langle T_i \rangle^3} - 9 - 6 CV_{T_i}^2 - 7 CV_{T_i}^4 \right)}{27 (3 + CV_{T_i}^2)^2} \\ &\quad + \frac{16 CV_\beta^2}{3(3 - CV_\beta^2)(3 + CV_{T_i}^2)}, \end{aligned} \quad (25)$$

where $CV_{T_i}^2$ and CV_β^2 denote randomness in the inter-division times (T_i) and partitioning errors (β), respectively, as quantified by their coefficient of variation squared. ■

The proof of Theorem 3 can be found in the Appendix. Interestingly, the mean cell size in (25) not only depends on the mean inter-division times $\langle T_i \rangle$, but also on its second-order moment $CV_{T_i}^2$. Thus, making the cell division times more random (i.e., increasing $CV_{T_i}^2$) will also lead to larger cells on average. Similar effects of $CV_{T_i}^2$ on mean gene expression levels have recently been reported in literature [24, 25]. Moreover, (26) shows that the magnitude of fluctuations in

cell size (CV_v^2) depend on T_i through its moments up to order three. Note that if $CV_\beta^2 = 0$ (no partitioning errors) and $T_i = \langle T_i \rangle$ with probability one (deterministic inter-division times), then $CV_v^2 = 1/27$. This non-zero value for CV_v^2 in the limit of vanishing noise sources represent variability in size from cells being in different stages of the deterministic cell cycle. Theorem 3 decomposes CV_v^2 into terms representing contributions from different noise sources. The terms from left to right in (26) represent contributions to CV_v^2 from i) Deterministic cell-cycle and ii) Random timing of division events and iii) Partitioning errors at the time of division. Assuming lognormally distributed T_i ,

$$\langle T_i^3 \rangle / \langle T_i \rangle^3 = (1 + CV_{T_i}^2)^3. \quad (27)$$

Substituting (27) in (26) and plotting CV_v^2 as a function of CV_β^2 and $CV_{T_i}^2$, reveals that stochastic variations in cell size are more sensitive to partitioning errors as compared to noise in the inter-division times.

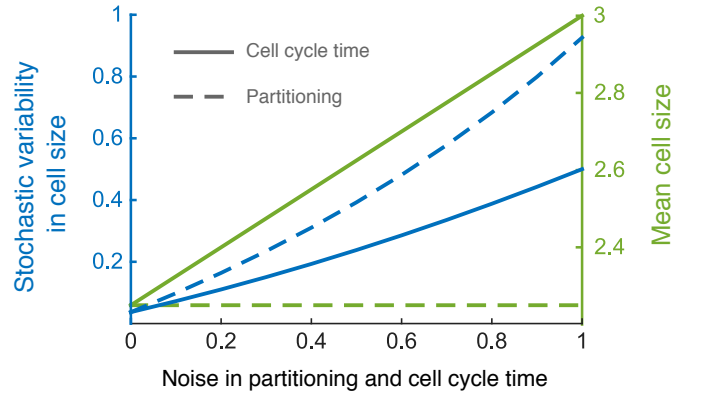


Fig. 2: Stochastic variation in cell size (blue) and mean cell size (green) as a function of $CV_{T_i}^2$ (noise in inter-division time) and CV_β^2 (error in partitioning of volume among daughters) for linear cell growth and a timer-based division mechanism. The mean cell size is dependent on $CV_{T_i}^2$ but independent of CV_β^2 . Fluctuations in cell size increase more rapidly with CV_β^2 than with $CV_{T_i}^2$.

In summary, our result show that appropriate regulation of growth rate by size (as seen in mammalian cells) can be an effective mechanism for achieving size homeostasis. We next consider a different class of models where size-based regulation is at the level division rather than growth.

IV. SIZE-DEPENDENT DIVISION RATE

In contrast to growth rate control, many organisms rely on size-dependent regulation of division rate for size homeostasis [26–30]. To analyze this strategy, we consider the SHS continuous dynamics (2) with a timer-dependent growth rate $\alpha(\tau)$, and a division rate $f(v, \tau)$ that now depends on size. The theorem below provides sufficient conditions on $f(v, \tau)$ for size homeostasis.

Theorem 4: Let there exist a non-decreasing function $g(\tau)$ and $p > 0$ such that

$$f(v, \tau) \geq g(\tau) v^p. \quad (28)$$

Moreover, the division rate for a sufficiently small cell size

$f_0(\tau) := \lim_{v \rightarrow 0} f(v, \tau)$ satisfies

$$\left\langle e^{\int_{y=0}^{T_i} \alpha_0(y) dy} \right\rangle > 2, \quad T_i \sim f_0(x) e^{-\int_{y=0}^x f_0(y) dy}. \quad (29)$$

Then, for the SHS given by (2) and (3)

$$0 < \lim_{t \rightarrow \infty} \langle v^l(t) \rangle < \left(\frac{l \langle \alpha(\tau) \rangle}{\langle g(\tau) \rangle (1 - \langle \beta^l \rangle)} \right)^{\frac{1}{p}}, \quad (30)$$

for $l \in \{1, 2, \dots\}$. ■

Proof of Theorem 4: Consider a newborn cell with a sufficiently small size at time $t = 0$. Then, based on Theorem 1, the mean size will grow over successive generations (and not go extinct) iff (29) holds. Based on the Dynkin's formula for (2)-(3), the time evolution of moments is given by

$$\frac{d \langle v^l \rangle}{dt} = l \langle \alpha(\tau) v^l \rangle - \langle f(v, \tau) v^l \rangle \langle 1 - \beta^l \rangle \quad (31)$$

Using (28), the fact that $\alpha(\tau)$ is a non-increasing function, while $g(\tau)$ is a non-decreasing function,

$$\frac{d \langle v^l \rangle}{dt} \leq l \langle \alpha(\tau) \rangle \langle v^l \rangle - \langle g(\tau) \rangle \langle v^{l+p} \rangle \langle 1 - \beta^l \rangle \quad (32)$$

Finally, using $\langle v^{l+p} \rangle \geq \langle v^l \rangle^{\frac{l+p}{l}}$ in (32) result in (30) at steady state. ■

Next, we show that different known strategies for size-dependent regulating of inter-division times are consistent with Theorem 4. A common example of size-dependent division is the “sizer strategy”, where a cell senses its size, and divides when a critical size threshold is reached [31–34]. Such as strategy can be implemented by

$$f(v, \tau) = \left(\frac{v}{\bar{v}} \right)^p \quad (33)$$

where \bar{v} and p are positive constant. A large enough p corresponds to division events occurring when size reaches \bar{v} . In contrast to the sizer strategy, many bacterial species use an “adder strategy”, where a cell divides after adding a fixed size from birth [35–38]. In the case of exponential growth (constant growth rate α), the adder strategy can be implemented by

$$f(v, \tau) = \left(\frac{v(1 - e^{-\alpha\tau})}{\bar{v}} \right)^p. \quad (34)$$

A large enough p would correspond to cells adding a fixed size \bar{v} between cell birth and division [39]. Both these division rates are consistent with the form of f required for size homeostasis in Theorem 4. We investigate the first two moments of v in more detail for the sizer strategy.

Using (31) for a constant growth rate α and division rate (33) results in the following moment dynamics

$$\frac{d \langle v^l \rangle}{dt} = l \alpha \langle v^l \rangle - \bar{v}^{-p} \langle v^{l+p} \rangle \langle 1 - \beta^l \rangle. \quad (35)$$

Let $\mu = [\langle v \rangle, \langle v^2 \rangle, \dots, \langle v^L \rangle]^T$ be a vector of moments up to order L , where L is the order of truncation. Using (35), the

time evolution of μ can be compactly written as

$$\frac{d\mu}{dt} = a + A\mu + C\bar{\mu}, \quad \bar{\mu} = [\langle v^{L+1} \rangle \dots \langle v^{L+p} \rangle]^T \quad (36)$$

for some vector a , matrices A and C , and $\bar{\mu}$ is the vector of higher order moments. Note that nonlinearities in the division rate lead to the well known problem of moment closure, where time evolution of μ depends on higher-order moments $\bar{\mu}$. Moment closure techniques that express $\bar{\mu} \approx \theta(\mu)$ are typically used to solve equations of the form (36). Here, we use closure schemes based on the derivative-matching technique [40–42], that yield analytical expressions for the steady-state moments. For example, $L = 2$ in (36) (second order of truncation) results in the following steady-state mean and coefficient of variation squared of cell size

$$\langle v \rangle \approx 2^{\frac{1}{p}} \alpha^{\frac{1}{p}} \bar{v} \left(\frac{3 - CV_\beta^2}{4} \right)^{\frac{p+1}{2p}}, \quad CV_v^2 \approx \left(\frac{4}{3 - CV_\beta^2} \right)^{\frac{1}{p}} - 1, \quad (37)$$

respectively. Intriguingly, (37) shows that the mean cell size decreases with increasing magnitude of partitioning error CV_β^2 . While the results from (37) are qualitatively consistent with moments obtained via Monte Carlo simulations, a much higher order of truncation is needed in (36) to get an exact quantitative match (Fig. 3).

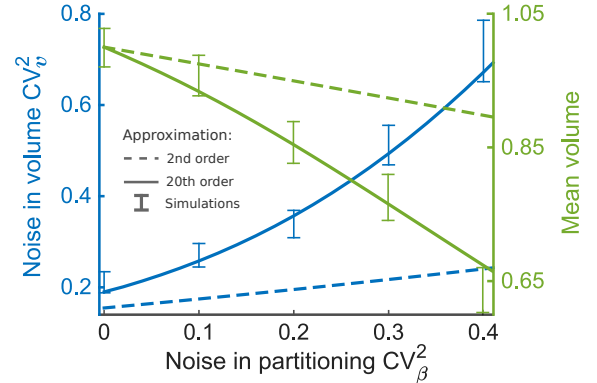


Fig. 3: Stochastic variation in cell size (blue) and mean cell size (green) as a function of CV_β^2 (error in partitioning of volume among daughters) for exponential cell growth and sizer-based division mechanism. The mean cell size decreases with increasing CV_β^2 , while noise in cell size increases with it. Results are shown for a 2nd (dashed) and a 20th (solid) order moment closure truncation, and compared with moments obtained by running a large number of Monte Carlo simulations. Errors bars show 95% confidence estimates.

V. CONCLUSION

Here we have used a phenomenological SHS framework to model time evolution of cell size (Fig. 1). The model is defined by three features: a growth rate $\alpha(v, \tau)$, a division rate $f(v, \tau)$, and a random variable $\beta \in (0, 1)$ that determines the reduction in size when division occurs. A key assumption was that α and f are monotone functions: with increasing size and cell-cycle progression, the growth rate decreases, and propensity to divide increases. Our main contribution was to identify sufficient conditions on α and f that prevent size extinction and also lead to bounded moments (Theorems 2 and 4). In essence, these conditions require the growth (division)

rate to decrease (increase) with cell size in a polynomial fashion.

We also analyzed two strategies for size homeostasis: i) Linear growth in size with timer-controlled divisions and ii) Exponential growth in size with size-controlled divisions. Analysis reveals that in the former strategy, the mean cell size is independent of volume partitioning errors at the time of mitosis. In contrast, the mean cell size decreases with increasing partitioning errors for size-controlled divisions. Moreover, stochastic variations in cell size are found to be highly sensitive to partitioning errors for both strategies (Fig. 2 and 3). This suggests that cells may use mechanisms to minimize volume mismatch among daughter cells. In summary, theoretical tools for SHS can provide fundamental understanding of regulation needed for size homeostasis. Future work will focus on coupling cell size to gene expression, and understanding how concentration of a given protein is maintained in growing cells [43–46].

APPENDIX: PROOF OF THEOREM 3

We prove Theorem 3 in the following steps: we use forward Kolmogorov equation to derive the equation describing the probabilistic evolution of timer τ . We use our derivation to calculate the probability distribution of timer and its moments. Next we use forward Kolmogorov equation again to derive the equation describing the joint probability distribution of timer τ and volume v and we calculate the steady-state conditional mean volume $\overline{\langle v|\tau \rangle}$; we denote the steady-state mean by $\overline{\langle \cdot \rangle}$. Lastly we uncondition $\overline{\langle v|\tau \rangle}$ to obtain $\overline{\langle v \rangle}$. We repeat the same steps for deriving $\overline{\langle v^2 \rangle}$.

A. The probability distribution of the timer τ

Using forward Kolmogorov equation for stochastic hybrid systems [47], the probability distribution of timer $p(\tau)$ at steady-state is described through

$$\frac{\partial p(\tau)}{\partial \tau} = -f(\tau)p(\tau), \quad \tau > 0, \quad (38)$$

where τ is the dummy variable for τ . We start our analysis by taking integral from both sides of (38)

$$\frac{\partial p(\tau)}{\partial \tau} = f(\tau)p(\tau) \Rightarrow p(\tau) = p_0 e^{-\int_0^\tau f(y)dy}, \quad (39)$$

where p_0 is a normalization constant. It can be shown that $p_0 = \overline{\langle f(\tau) \rangle}$

$$\begin{aligned} \overline{\langle f(\tau) \rangle} &= p_0 \int_0^\infty f(\tau) e^{-\int_0^\tau f(y)dy} d\tau \\ &\Rightarrow \overline{\langle f(\tau) \rangle} = p_0 \left(-e^{-\int_0^\tau f(y)dy} \right)_0^\infty \Rightarrow \overline{\langle f(\tau) \rangle} = p_0. \end{aligned} \quad (40)$$

Hence $p(\tau)$ can be written as

$$p(\tau) = \frac{1}{\langle T_i \rangle} e^{-\int_0^\tau f(y)dy}. \quad (41)$$

Moreover moments at steady state of τ can be calculated from the probability distribution

$$\overline{\langle \tau \rangle} = \frac{1}{\langle T_i \rangle} \int_0^\infty \tau e^{-\int_0^\tau f(y)dy} d\tau. \quad (42)$$

Note that from equation (4) in the main text we can calculate the second order moment $\langle T_i^2 \rangle$ as

$$\langle T_i^2 \rangle = \int_0^\infty x^2 f(x) e^{-\int_0^x f(y)dy} dx, \quad (43)$$

integrating by parts results in

$$\langle T_i^2 \rangle = 2 \int_0^\infty x e^{-\int_0^x f(y)dy} dx. \quad (44)$$

Hence by a change of variables in (44) and using (42) we have

$$\overline{\langle \tau \rangle} = \frac{\langle T_i^2 \rangle}{2\langle T_i \rangle}. \quad (45)$$

Using similar analysis results in

$$\overline{\langle \tau^2 \rangle} = \frac{\langle T_i^3 \rangle}{3\langle T_i \rangle}. \quad (46)$$

B. Mean of cell volume $\overline{\langle v \rangle}$

From the forward Kolmogorov equation, the joint probability distribution of the timer and the volume $p(\tau, v)$ at steady-state is given by

$$\frac{\partial p(\tau, v)}{\partial \tau} + \frac{\partial}{\partial v} (kp(\tau, v)) = -f(\tau)p(\tau, v), \quad \tau > 0. \quad (47)$$

The conditional mean volume $\overline{\langle v|\tau \rangle}$ can be written as

$$\overline{\langle v|\tau \rangle} \equiv \overline{\langle v|\tau = \tau \rangle} = \frac{1}{p(\tau)} \int_0^{+\infty} vp(\tau, v) dv. \quad (48)$$

Taking derivative with respect to τ from (48) results in

$$\begin{aligned} \frac{\partial \overline{\langle v|\tau \rangle}}{\partial \tau} &= -\frac{\frac{\partial p(\tau)}{\partial \tau}}{p^2(\tau)} \int_0^{+\infty} vp(\tau, v) dv \\ &\quad + \frac{1}{p(\tau)} \int_0^{+\infty} v \frac{\partial p(\tau, v)}{\partial \tau} dv. \end{aligned} \quad (49)$$

To calculate $\frac{\partial \overline{\langle v|\tau \rangle}}{\partial \tau}$ we need expressions for $\frac{\partial p(\tau, v)}{\partial \tau}$ and $\frac{\partial p(\tau)}{\partial \tau}$. Substituting these expressions from (47) and (38) in (49) and performing some algebraic manipulations gives

$$\frac{\partial \overline{\langle v|\tau \rangle}}{\partial \tau} = k \quad (50)$$

Hence the mean volume given the timer is given by

$$\overline{\langle v|\tau \rangle} = k\tau + \overline{\langle v|\tau = 0 \rangle}. \quad (51)$$

To calculate $\overline{\langle v|\tau = 0 \rangle}$ we use equation (3) in the main text. Note that in the time of division $\tau \mapsto 0$, hence in the time of division $v|\tau = T_i \mapsto v|\tau = 0$. Given the fact that the volume before and after division are related via (3), $v|\tau = 0$ is equal to $\beta v|\tau = T_i$, $i = \{1, 2, \dots\}$. Hence the mean volume after division is related to the mean volume right before division as

$$\langle v|\tau = 0 \rangle = \langle \beta \rangle \langle v|\tau = T_i \rangle, \quad i = \{1, 2, \dots\}. \quad (52)$$

Thus the mean volume after divisions can be written as

$$\overline{\langle v|\tau = 0 \rangle} = \langle \beta \rangle \overline{\langle v|\tau = \langle T_i \rangle \rangle}. \quad (53)$$

By substituting (53) in (51) we have

$$\overline{\langle v | \tau = 0 \rangle} = \frac{\langle \beta \rangle}{1 - \langle \beta \rangle} \langle T_i \rangle. \quad (54)$$

Thus equation (51) can be written as

$$\overline{\langle v | \tau \rangle} = k\tau + k \frac{\langle \beta \rangle}{1 - \langle \beta \rangle} \langle T_i \rangle. \quad (55)$$

The mean volume $\overline{\langle v \rangle}$ can be derived by multiplying $p(\tau)$ from (41) to equation (55), taking integral from both sides, and using (45)

$$\begin{aligned} \overline{\langle v \rangle} &= k \frac{\langle T_i^2 \rangle}{2 \langle T_i \rangle} + k \frac{\langle \beta \rangle}{1 - \langle \beta \rangle} \langle T_i \rangle \Rightarrow \\ \overline{\langle v \rangle} &= k \langle T_i \rangle \frac{1 + \langle \beta \rangle}{2(1 - \langle \beta \rangle)} + \frac{k \langle T_i \rangle CV_{T_i}^2}{2}. \end{aligned} \quad (56)$$

C. The second order moment of the volume $\overline{\langle v^2 \rangle}$

The sketch of the proof for the second order moment is similar to the mean of the volume. We start by deriving the conditional moment $\overline{\langle v^2 | \tau \rangle}$, and then we uncondition it to calculate $\overline{\langle v^2 \rangle}$; the conditional second order moment of the volume is defined as

$$\overline{\langle v^2 | \tau \rangle} \equiv \frac{1}{p(\tau)} \int_0^{+\infty} v^2 p(\tau, v) dv. \quad (57)$$

Taking derivative with respect to τ from (57) results in

$$\begin{aligned} \frac{\partial \overline{\langle v^2 | \tau \rangle}}{\partial \tau} &= - \frac{\partial p(\tau)}{p^2(\tau)} \int_0^{+\infty} v^2 p(\tau, v) dv \\ &+ \frac{1}{p(\tau)} \int_0^{+\infty} v^2 \frac{\partial p(\tau, v)}{\partial \tau} dv. \end{aligned} \quad (58)$$

Substituting (47) and (38) in (58) yields

$$\frac{\partial \overline{\langle v^2 | \tau \rangle}}{\partial \tau} = 2k \overline{\langle v | \tau \rangle} \Rightarrow \frac{\partial \overline{\langle v^2 | \tau \rangle}}{\partial \tau} = 2k^2 \tau + 2k^2 \frac{\langle \beta \rangle}{1 - \langle \beta \rangle} \langle T_i \rangle. \quad (59)$$

Thus $\overline{\langle v^2 | \tau \rangle}$ can be written as

$$\overline{\langle v^2 | \tau \rangle} = k^2 \tau^2 + 2k^2 \frac{\langle \beta \rangle}{1 - \langle \beta \rangle} \langle T_i \rangle \tau + \overline{\langle v^2 | \tau = 0 \rangle}. \quad (60)$$

In order to calculate $\overline{\langle v^2 | \tau = 0 \rangle}$ we use equation (3) in the main article

$$\overline{\langle v^2 | \tau = 0 \rangle} = \langle \beta^2 \rangle \overline{\langle v^2 | \tau = \langle T_i \rangle \rangle}. \quad (61)$$

Using (61) in (60) results

$$\overline{\langle v^2 | \tau = 0 \rangle} = \frac{k^2 \langle T_i \rangle^2 \langle \beta \rangle^2 (1 + CV_\beta^2) (\frac{1 + \langle \beta \rangle}{1 - \langle \beta \rangle}) + CV_{T_i}^2}{1 - \langle \beta \rangle^2 (1 + CV_\beta^2)}. \quad (62)$$

Hence $\overline{\langle v^2 \rangle}$ can be derived by unconditioning (60), and using (46)

$$\overline{\langle v^2 \rangle} = k^2 \frac{\langle T_i^3 \rangle}{3 \langle T_i \rangle} + k^2 \frac{\langle \beta \rangle}{1 - \langle \beta \rangle} \langle T_i^2 \rangle + \overline{\langle v^2 | \tau = 0 \rangle}, \quad (63)$$

in which initial condition is given by (62). By having the

second order moment one can calculate the noise by deriving CV_v^2 ; for example by selecting $\langle \beta \rangle = \frac{1}{2}$, noise is quantified as equation (28) in the main text.

REFERENCES

- [1] A. C. Lloyd, "The Regulation of Cell Size," *Cell*, vol. 154, pp. 1194–1205, 2013.
- [2] M. Campos, I. V. Surovtsev, S. Kato, A. Paintdakhi, B. Beltran, S. E. Ebmeier, and C. Jacobs-Wagner, "A constant size extension drives bacterial cell size homeostasis," *Cell*, vol. 159, pp. 1433–1446, 2014.
- [3] D. J. Kiviet, P. Nghe, N. Walker, S. Boulineau, V. Sunderlikova, and S. J. Tans, "Stochasticity of metabolism and growth at the single-cell level," *Nature*, vol. 514, pp. 376–379, 2014.
- [4] L. Robert, M. Hoffmann, N. Krell, S. Aymerich, J. Robert, and M. Doumic, "Division in *Escherichia coli* is triggered by a size-sensing rather than a timing mechanism," *BMC Biology*, vol. 12, p. 17, 2014.
- [5] T. P. Miettinen and M. Björklund, "Mevalonate Pathway Regulates Cell Size Homeostasis and Proteostasis through Autophagy," *Cell Reports*, vol. 13, pp. 2610–2620, 2015.
- [6] K. M. Schmoller and J. M. Skotheim, "The Biosynthetic Basis of Cell Size Control," *Trends in Cell Biology*, vol. 25, pp. 793–802, 2015.
- [7] S. Jun and S. Taheri-Araghi, "Cell-size maintenance: universal strategy revealed," *Trends in Microbiology*, vol. 23, pp. 4–6, 2015.
- [8] S. Taheri-Araghi, S. Bradde, J. T. Sauls, N. S. Hill, P. A. Levin, J. Paulsson, M. Vergassola, and S. Jun, "Cell-size control and homeostasis in bacteria," *Current Biology*, vol. 25, pp. 385–391, 2015.
- [9] Y. Tanouchi, A. Pai, H. Park, S. Huang, R. Stamatov, N. E. Buchler, and L. You, "A noisy linear map underlies oscillations in cell size and gene expression in bacteria," *Nature*, vol. 523, pp. 357–360, 2015.
- [10] A. Zaritsky and C. L. Woldringh, "Chromosome replication, cell growth, division and shape: a personal perspective," *Microbial Physiology and Metabolism*, vol. 6, p. 756, 2015.
- [11] A. Zaritsky, "Cell-Shape Homeostasis in *Escherichia coli* Is Driven by Growth, Division, and Nucleoid Complexity," *Biophysical Journal*, vol. 109, pp. 178–181, 2015.
- [12] J. T. Sauls, D. Li, and S. Jun, "Adder and a coarse-grained approach to cell size homeostasis in bacteria," *Current Opinion in Cell Biology*, vol. 38, pp. 38–44, 2016.
- [13] F. R. Cross and J. G. Umen, "The *Chlamydomonas* cell cycle," *The Plant Journal: For Cell and Molecular Biology*, vol. 82, pp. 370–392, 2015.
- [14] Y. Zegman, D. Bonazzi, and N. Minc, "Measurement and manipulation of cell size parameters in fission yeast," *Methods in Cell Biology*, vol. 125, pp. 423–436, 2015.
- [15] S. Banerjee, K. Lo, T. Kuntz, M. K. Daddysman, A. R. Dinner, and N. F. Scherer, "Crossover in the dynamics of cell wall growth controls bacterial division times," *bioRxiv*, p. 047589, 2016. <http://dx.doi.org/10.1101/047589>.

- [16] A. Marantan and A. Amir, "Stochastic modeling of cell growth with symmetric or asymmetric division," *arXiv:1602.01848 [q-bio]*, 2016. <http://arxiv.org/abs/1602.01848>.
- [17] S. M. Ross, "Reliability theory," in *Introduction to Probability Models*, pp. 579 – 629, Academic Press, tenth ed., 2010.
- [18] A. Tzur, R. Kafri, V. S. LeBleu, G. Lahav, and M. W. Kirschner, "Cell Growth and Size Homeostasis in Proliferating Animal Cells," *Science*, vol. 325, pp. 167–171, 2009.
- [19] R. Kafri, J. Levy, M. B. Ginzberg, S. Oh, G. Lahav, and M. W. Kirschner, "Dynamics extracted from fixed cells reveal feedback linking cell growth to cell cycle," *Nature*, vol. 494, pp. 480–483, 2013.
- [20] M. B. Ginzberg, R. Kafri, and M. Kirschner, "On being the right (cell) size," *Science*, vol. 348, p. 1245075, 2015.
- [21] J. P. Hespanha and A. Singh, "Stochastic models for chemically reacting systems using polynomial stochastic hybrid systems," *International Journal of Robust and Nonlinear Control*, vol. 15, pp. 669–689, 2005.
- [22] M. Finkelstein, "Failure Rate and Mean Remaining Lifetime," in *Failure Rate Modelling for Reliability and Risk*, Springer Series in Reliability Engineering, pp. 9–44, Springer, 2008.
- [23] I. Conlon and M. Raff, "Differences in the way a mammalian cell and yeast cells coordinate cell growth and cell-cycle progression," *Journal of Biology*, vol. 2, p. 7, 2003.
- [24] M. Soltani, C. Vargas, D. Antunes, and A. Singh, "Decomposing variability in protein levels from noisy expression, genome duplication and partitioning errors during cell-divisions," *To Appear in PLOS Computational Biology*, 2015. <http://arxiv.org/abs/1509.04559>.
- [25] D. Antunes and A. Singh, "Quantifying gene expression variability arising from randomness in cell division times," *Journal of Mathematical Biology*, vol. 71, pp. 437–463, 2014.
- [26] S. Cooper, "Distinguishing between linear and exponential cell growth during the division cycle: Single-cell studies, cell-culture studies, and the object of cell-cycle research," *Theoretical Biology and Medical Modelling*, vol. 3, p. 10, 2006.
- [27] S. Cooper, "Schizosaccharomyces pombe grows exponentially during the division cycle with no rate change points," *FEMS Yeast Research*, vol. 13, pp. 650–658, 2013.
- [28] L. Robert, M. Hoffmann, N. Krell, S. Aymerich, J. Robert, and M. Doumic, "Division in Escherichia coli is triggered by a size-sensing rather than a timing mechanism," *BMC Biology*, vol. 12, p. 17, 2014.
- [29] M. Osella, E. Nugent, and M. C. Lagomarsino, "Concerted control of Escherichia coli cell division," *Proceedings of the National Academy of Sciences*, vol. 111, pp. 3431–3435, 2014.
- [30] M. H. Rahman, M. R. Ahmad, M. Takeuchi, M. Nakajima, Y. Hasegawa, and T. Fukuda, "Single Cell Mass Measurement Using Drag Force Inside Lab-on-Chip Microfluidics System," *IEEE Transactions on NanoBioscience*, vol. 14, pp. 927–934, 2015.
- [31] J. J. Tyson and O. Diekmann, "Sloppy size control of the cell division cycle," *Journal of Theoretical Biology*, vol. 118, pp. 405 – 426, 1986.
- [32] J. J. Turner, J. C. Ewald, and J. M. Skotheim, "Cell Size Control in Yeast," *Current Biology*, vol. 22, pp. R350–R359, 2012.
- [33] K. Z. Pan, T. E. Saunders, I. Flor-Parra, M. Howard, and F. Chang, "Cortical regulation of cell size by a sizer cdr2p," *eLife*, vol. 3, p. e02040, 2014.
- [34] K. M. Schmoller, J. J. Turner, M. Koivomagi, and J. M. Skotheim, "Dilution of the cell cycle inhibitor Whi5 controls budding-yeast cell size," *Nature*, vol. 526, pp. 268–272, 2015.
- [35] A. Amir, "Cell size regulation in bacteria," *Physical Review Letters*, vol. 112, p. 208102, 2014.
- [36] M. Deforet, D. van Ditmarsch, and J. B. Xavier, "Cell-size homeostasis and the incremental rule in a bacterial pathogen," *Biophysical Journal*, vol. 109, pp. 521–528, 2015.
- [37] A. Fievet, A. Ducret, T. Mignot, O. Valette, L. Robert, R. Pardoux, A. R. Dolla, and C. Aubert, "Single-cell analysis of growth and cell division of the anaerobe Desulfovibrio vulgaris Hildenborough," *Frontiers in Microbiology*, vol. 6, p. 1378, 2015.
- [38] I. Soifer, L. Robert, and A. Amir, "Single-cell analysis of growth in budding yeast and bacteria reveals a common size regulation strategy," *Current Biology*, vol. In Press, Corrected Proof version, 2016.
- [39] K. R. Ghusinga, C. A. Vargas-Garcia, and A. Singh, "A mechanistic first-passage time framework for bacterial cell-division timing," *arXiv:1512.07864 [q-bio]*, 2015. <http://arxiv.org/abs/1512.07864>.
- [40] A. Singh and J. P. Hespanha, "Stochastic analysis of gene regulatory networks using moment closure," in *Proc. of the 2007 Amer. Control Conference, New York, NY*, 2006.
- [41] A. Singh and J. P. Hespanha, "Models for multi-specie chemical reactions using polynomial stochastic hybrid systems," in *IEEE Conference on Decision and Control*, 2005.
- [42] A. Singh and J. P. Hespanha, "Approximate moment dynamics for chemically reacting systems," *IEEE Transactions on Automatic Control*, vol. 56, pp. 414–418, 2011.
- [43] M. Soltani and A. Singh, "Cell-cycle coupled expression minimizes random fluctuations in gene product levels," *arXiv:1605.02251 [q-bio]*, 2016. <http://arxiv.org/abs/1605.02251>.
- [44] O. Padovan-Merhar, G. P. Nair, A. G. Bialesch, A. Mayer, S. Scarfone, S. W. Foley, A. R. Wu, L. S. Churchman, A. Singh, and A. Raj, "Single mammalian cells compensate for differences in cellular volume and DNA copy number through independent global transcriptional mechanisms," *Molecular Cell*, vol. 58, pp. 339–352, 2015.
- [45] V. Shahrezaei and S. Marguerat, "Connecting growth with gene expression: of noise and numbers," *Current*

Opinion in Microbiology, vol. 25, pp. 127–135, 2015.

- [46] S. Marguerat and J. Bähler, “Coordinating genome expression with cell size,” *Trends in Genetics*, vol. 28, pp. 560–565, 2012.
- [47] N. G. V. Kampen, *Stochastic Processes in Physics and Chemistry*. Amsterdam: North Holland, 1992.